Relationship between different Anthropometric and Cardiovascular risk factors in Lean Vs Obese Women with PCOS.

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Introduction:

Polycystic Ovary Syndrome (PCOS) is a complex genetic disease found in women during the reproductive age. The main characterstic features of PCOS are excess androgen production, chronic oligo/anovulation and polycystic ovaries are seen in ultrasound. Clinically these women are present with irregular menses, hirustism, acne & alopecia, elevated LH;(Leutinizing Hormone), FSH; (Follicle Stimulating Hormone) ratio along with insulin and Androgen excess.The syndrome also develops a great risk of development of Type 2 Diabetesand cardiovascular diseases in later stage of life. In 50-70%affected womem with PCOS, insulin resistance was observed [2]. Central Obesity was also observed in the patients of PCOS which aggrevates the symptoms [3].There are many pathways that explains the etiology of PCOS as a good example of it like-regulatory pathways of gonadotropin action, the metabolic synthesis, pathways regulating body weight and the insulin signaling pathway. Various genes from these different pathways have been tested in the form of candidate gene for PCOS. In different research studies, as per the proposed diagnostic criteria, the prevalence of PCOS has been reported 6%-19% [4]. The other factors like sedentary life style, environmental or acquired factors, when interact with multiple genetic factors related to hyperandrogenism, can also increase the dysregulation of androgen synthesis. This does not allow the ovarian follicle to grow as dominion follicle and results in oligo / anovulation and PCOS [5].

The pathogenesis of PCOS is tremendous as it includes the contributions from insulin resistance, misfunctioning of the adipose tissues, abnormal production of steroids and hypothalamic-pituitary-ovarian dysregulation. PCOS which is the result of genetic and epigenetic factors, and there is dysregulation of these varied system, possibly beginning in vitro, opens new avenues for research investigation and further action plan.

OBJECTIVES OF THE STUDY

- 01. The aim of this study is to do the comparative analysis of the anthropometric and cardiovascular risk factors between the lean and obese women having PCOS.
- 02. To measure biochemical and hormonal parameters in PCOS subjects and healthy controls. Analysis of biochemical and hormonal profiles.
- 03. To analyze various CVD (Cardiovascular) Bio-markers, Inflammatory markers such a C-reactive protein (CRP), Interleukin-6 (IL-6), Fibrinogen and Tumornecrosis factor (TNF-α) etc.
- 4. Association of various metabolic markers such as Total Cholesterol, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Triglycerides (TG's), Vitamin D, Adeponein etc.
- 5. Fasting glucose, systolic and diastolic BP, Smoking habits.

SCOPE OF STUDY:-

1.The result of this study will help in understanding PCOS and its impact in women's quality of life.

2.The study highlights PCOS challenge, and the support rendered by Nonprofit organization for women and girls with PCOS. It has been good to know and experience the true state of women's health.

3.The study provides useful and productive suggestions to PCOS subjects and overcome the feeling of being alone.

4.PCOS is considered to be linked with reproductive morbidity and there is increased risk for endometrial cancer. Prior diagnosis is especially important because it is also presumed that PCOS may increase Cardiovascular and Metabolic risks.

5.The studies show that Polycystic ovary syndrome is associated with hormonal, metabolic and psychosocial disorder that finally impacts quality of life of the patient. Another important aspect to treat these patients must be through holoistic approach which help them to deal with the emotional stress. This aspect is often overlooked with the patients of Polycysticovarian syndrome.

6.Polycystic Ovary Syndrome is a disease which is not considered seriously in the society and is often neglected.

HYPOTHESIS:-

1. In the present study on patients with Polycystic Ovarian Syndrome (PCOS), it is hypothesized that whether increased risk for Cardiovascular disorder is related more to Obesity than PCOS in patients.
2. Polycystic ovary Syndrome (PCOS) was hypothesized as a result of functional ovarian hyperandrogenism (FOH) due to dysregulation of androgen secretion.

REVIEW OF LITERATURE

The till date research done in this field; research scholar has consulted the related literature on this vibrant topic. The scholar has searched almost every source like research journals, periodicals, encyclopedia's, books of various kinds and websites of PCOS related research topics in the internet. However, the research scholar has also gone through literatures of allied studies for preparing the proper hypothesis and outcome of the study. Brief outlines on reviews of the studies conducted so far has been included and considered in present study.

In very early research in fifth century, the distinguished reproductive features of PCOS were noted by Hippocrates in the ***Bew C (Hanson 1975).***MORGAGNI described a74 year old woman with obesity characterstic and android aspect ***(Valde Obese et al Virili aspect)***. It was reported by ***Archad and Thiers***in (1921) that there is some relation of diabetes mellitus and clinical signs of androgen excess in postmenopausal Women- the so called "Archad, Thiers Syndrome". So many research was carried out and published over years, but more focus was aroused in1935 when it was found that bilateral polycystic Ovaries were related with a clinical syndrome consisting of irregularity in menstrual cycle featuring amenorrhea ***(Stein and Leventhal 1935)***. The condition was termed on their names as the Stein and Leventhal Syndrome for long time. The link with skin lesion, *Acanthosisnigricans*, with diabetes mellitus and hyperandrogenism was investigated and reported to occur frequently in women by ***Keirland et al,. (1947).***In another study***Jean Vague (1947)*** from the University of Marseille defined the term" Android obesity" and explained the abdominal fat accumulation observed in male pattern of body fat distribution. He tried to explore whether this type of body fat adiposity was having link with increased cardiovascular disease and diabetes risk. In another study by ***Kissebehat et***

al., (1982) reported that women with obesity at upper body part were found resistant to insulin. The increased androgen production rates were also observed in these women.***(Kirschner et al, 1990, Brown and Winkellman(1968)*** noted that it was genetic basis and insulin- resistant diabetes mellitus, as reported of affected sisters***(Barnes et al., (1974),***and identical twins who were also having acromegaloid features ***(Colle et al, 1979)***.Although it was suspected as early as 1962 that there is a vast area of clinical presentation of PCOS, the concept of PCOS and simultaneously normal level of LH concentrations was not observed by researchers till 1976***(Reber et al 1976).***

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A group of scientists started working to explore the association of hyperandrogenism, insulin resistance and Acanthosisnigaricans when Kahn and Colleagues ***(Kahn et al. 1976)*** described the type A Syndrome, which was a distinct disorder affecting young age girls. These girls were having extreme resistance towards insulin with diabetes mellitus, virilized (i, e temporal balding, increased muscle bulk clitoromegaly and as well as striking Acanthosis nigricans. In an study on postmenopausal women with Acanthosisnigricans and autoimmune disease, extreme insulin resistance syndrome was observed and they termed it Type B syndrome and reported that it was due to the androgenous anti insulin receptor antibodies ***(Rabilly et al., 1991).***Since, the report by ***Bhergen etal.,(1780)*** that PCOS was associated with hyperinsulinimia, a correlation was developed with syndrome and major metabolic as well as reproductive morbities. Furthermore a relation was also observed between women with typical PCOS had *Acanthosisnigricans*, and the women with the rare syndromes of extreme insulin resistance***(Dunaif et al., 1985,Flier et al., 1985).***They further reported that women with the common hyper androgenic disorder had basal and glucose stimulated hyperinsulinimia compared with control women of same weight and suggested the tendency of insulin resistance. The ultra -sonographic finding of polycystic ovaries was discussed for the first time by ***Swanson et al., (1981).*** A new definition for the ultra-sonographic appearance of PCOS was introduced by Adams and Coworkers in 1985 as one of the major diagnostic criterion of PCOS ***(Adam S et al., 1985).***This definition has been extensively used after that.

2.2 : DEFINITION OF POLYCYSTIC OVARIAN SYNDROME:-

From the time it was reported in 1953 until 1990, there was no clear cut tool for diagnosing polycystic ovarian syndrome. In 1990, a conference was organized by National Institute of Health (NIH) to finally established a criterion for the diagnosis of PCOS. As per the NIH criteria PCOS is defined as irregularity in menstrual cycle, oligomennorhea or anovulation, hyperandrogenism (except androgen activity, clinical alterations). In the exclusion category, hypothalamic, amenorrhea, hyperptolactinae, hyperandrogenism- insulin resistance acanthosisnigricans (Hairan) syndrome were considered.

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Till 2003 before the Rotterdam conference was held, Hyperplasia (CAH) Cushing Syndrome, tumour with androgen secretion, exogenous androgen overdose and hypo/hyperthyrosis) ***(Azziz et al., 2006)*** were the main diagnostic tool used for PCOS. In 2003, a new criterion was introduced by the Rotterdam consensus for the definition of PCOS. Ultrasound detected Ovarian morphology was considered world vide by researchers and clinicians ***(Merino et al., 2011).*** This consensus was organized by American Society for Reproductive Medicine (ASRM) and also by the European Society of Human Reproduction and Embryology (ESHRE). Based on the consensus, whenever any two criteria from the previous three criteria: - 1.Excess androgen activity, 2.Oligo- and or anovulation and 3. Ultrasound detected Polycystic ovarian morphology are present , it is diagnosed as PCO. ASRM/ ESHRE consensus defines polycystic ovaries.In this condition, at least one of the ovaries has at least either 12 or more follicles measuring 2.9mm in diameter, the scan should be repeated at a time of ovarian/ quiescence for calculation of volume and area. While comparing, it was concluded that, The Rotterdam definition was much broader as compared to that of NIH consensus available, which included patients without androgen excess.

2.3: EPIDEMIOLOGY OF PCOS: -

4 to 10 % of fertile women are affected by PCOS around the world ***(Heart et al.,2011)***, but some researchers claim the overall rate to be in the range of 4 to 25% (Homberg 2002). Difference in the openion on rates may be due to the different criteria used for the diagnosis of the syndrome different races and ethnicities. ***Kuaffman et al., (2002)*** observed that in Mexican American women who were suffering from PCOS had

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a higher prevalence of IR as compared to the White American Women under same experimental conditions. ***Baleen et al., (2004)*** reported that more severe symptoms were observed in patients of South Asian origin at young age with advanced incidence of IR as compared to Caucasian British women. A higher risk for PCOS and IR was observed in Asian populations, however the minimum prevalence of metabolic syndrome was observed among Chinese women as compared to patients of other countries ***(NIET et al., 2009).***

PCOS in India and Subcontinent:-

Rapid urbanization and changes in life style in many developing countries like India, is causing an increase in many complex diseases like PCOS, Cardiovascular Disease and Diabetes. Prevalence of PCOS is also rapidly rising among Indians also. Estimates of PCOS in migrant Indians have been reported approximately 52%. About 37% among the North Indian Women have been estimated to suffer from PCOS ***(Allahbadia and Merchant, 2008;(Dasgupta et al., 2008)***. Although, there are limited studies of PCOS have been carried out in India. Most of the prevalence studies conducted in schools among adolescents reported the prevalence of PCOS as 9.13% to 36% ***(Nidhi et al., 2011; Nair et al., 2012)***. Some studies conducted in India schools have been confined to clinical dimensions of PCOS. Other studies on Indians are from South Asia and emigrant Indians. ***Maitro et al., (2004)*** conducted mutational analysis of CYP1A1 and leptin as genetic determinants of hyperandrogenecity and obesity in PCOS. The same group has shown the existence of dyslipidemia associated with Cardiovascular risks and obesity in PCOS.

Kalra et al., (2006) and his co-workers have shown that insulin resistance is associated with the prevalence of type 2 diabetes. Another study, a greater insulin resistance was observed in South Indian women having reproductive abnormalities of PCOS and intima media thickness that would lead to risks of vascular diseases ***(Sudaram et al., 2003***). Asian Indians being more resistant to insulin may exhibit higher predisposition to metabolic syndrome also at an earlier stage.

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Although Indian population contributes greatly to the world's total population genetic studies related to PCOS is scantly and therefore it has become imperative to explore the etiology (both environmental and genetic) in the manifestation of PCOS.

2.4 CLINICAL FEATURES:-

In the clinical scenariotill date, PCOS is conventionally viewed as" a young lady, probably who is obese presenting with features of hirustism, oligomennorhea and infertility."

2.4.1 MENSTRUAL IRREGULARITIES:-

Chronic anovulation often presents as oligomennorhea, amenorrhea, dysfunctional uterus bleeding and / or infertility oligomennorhea is commonly termed as a menstrual interval of days and on other hand amenorrhea is defined as the absence if menstrual bleeding >90 days. The menstrual irregularity observed in PCOS is chronic and can manifest in several different ways. Between 20- 30% of women with the syndrome PCOS with a history of Amenorrhea (normal menstrual cycle). Primary amenorrhea (mensuration never starting) is an uncommon manifestation of PCOS which ranges from 1.4 to 14% as per the different Cohorts ***(Franks, 2002; Rachmiel et al, 2008).***

2.4.2 HYPERANDROGENISM:-

It is the second defining characteristic of PCOS. The reported symptoms of hyperandrogenism are hirustism, acne, and/or androgen alopecia. However, it is also mentioned that androgen alopecia is quite a poor marker to establish enndrogen excess (***Rotterdam, ESHRE/ASRM-Sponsored, PCOS, Consensus, Workshop, Group, 2004)***.

2.4.2.1 HIRSUTISM:-

In this condition, male like pattern is observed in woman. Excessive hair growth is a major symptom in PCOS. Mostly 17 and 83% of women with PCOS are also suffered from hirustism. Ferriman Gallway described the method of clinical assessment of the stage of hirustism on the basis of the visual scoring (***Ferriman and Gallway, 1961).***

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This scale quantifies the presence of terminal hair over nine different body parts: the upper lip, lower and upper abdomen, chest, chin, thighs, both upper & lower back and on the upper arms. Degree of hirustism is scored on a scale ranging from 0 to 4 on these sites.

Azziz et al., (2004) defined hirustism as a FG score of 6 or higher, on the other hand ***Asuncion et al., (2000)*** described a FG score of 8 or higher. Also hirustism score depends a lot on the ethnicity of the population. It has been observed that in the patients of hailing from Asia in the United States lower degree of hirustism was shown (Hull, 1987).Pakistani women living in United Kingdom have high hirustism.(***Wijey- Aratne et al., 2002***)

2.4.2.2:- ACNE VULGARIS:-

Acne is a disorder of the Pilosebaceous. It may not be easy to demarcate between normal pubertal acne in an adolescent with PCOS.A proper investigation is required for an adolescent female with moderate or severe acne weather she is suffering from acne or not. Furthermore, the persistence of acne into adulthood is unusual and need proper attention. Prevalence of Acne is approximately 15- 25% in PCOS patients ***(Azziz et al., 2006).*** Androgens play an important role in the development of acne. In the sebaceous gland, testosterone is converted to a more potent androgen, DHT with the help of enzyme 5 Alpha reductase, which in turn increases sebum production from sebaceous glands. Inflammation, genetic, environmental factors and bacterial infections like propinibacterium acne are involved in the etiology of acne.

2.4.2.3:-ALOPECIA:-

In the PCOS females, excess hair growth on the face and body and another contrary problem of thinning scalp hair, known as androgenic alopecia is observed simultaneously. But the best part is that in women with PCOS, the hair follicle remains alive. These live hair follicles increase the chances of better results in hair loss therapy and new hair growth. The prevalence of alopecia in women with PCOS is reported from 5 to 50% ***(Azziz et al., 2006)***

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2.4.2.4:-ACANTHOSIS NIGRICANS:-

Acanthosisnigricans on physical examination is a sign of IR which is characterized by abnormal thickening of the skin, especially in body increases (commonly at neck, armpit and groins). During clinical examination it is vital to look for the signs of insulin resistance.

2.4.2.5:-BIOCHEMICAL SIGNS:-

Women with PCOS have also evidence of biochemical hyperandrogeneamia and high levels of testosterone in circulation serves as a diagnostic marker for androgen excess. Testosterone is circulated as free and bound from with SHBG and albumin. The value of testosterone is measured primarily so that other causes of androgen excess are excluded. The measurement of free testosterone or FAI may also be used for assessing for hyperandrogeneamia. The PCOS is also associated with other disorders like diabetes, obesity, insulin resistance, hyperinsulinemia, hypertension and disturbed lipid profile ***(Azziz et al., 2006).*** For each woman suffering from PCOS, sign and symptoms may be different and may also vary over time ***(Azziz et al., 2006)***. Weight gain in the patient of PCOS, usually an aggravates the symptoms, while weight loss usually improves the symptoms and other endocrine and metabolic disturbances associated with this ***(Clark et al., 1995) .***

Tatboltand et al., in 1995, reported lipid abnormalities were observed in PCOS females.

A higher level of total cholesterol, TG and LDL and lower level of HDL was observed.

A study conducted to determine the role of circulating TNFα and the relationship of TNFα with insulin levels, body weight, or both in women suffering from PCOS ***(Gonzalez F et al. 1999)***. It was concluded that serum TNFα is increased in normal weight women and is even higher in obese individuals irrespective of whether the woman is suffering from PCOS or not. Increased circulating TNFα may encourage insulin resistance in the obese patient, which can also result and promote hyperandrogenism in PCOS women with higher weight. It is again a question of further research whether this same pattern is also observed in case of normal weight women with PCOS.

Evanthiadiamanti- Kandarakis et al, in 2006 reported that higher plasma levels were observed of hs-CRP(mg/ l) in PCOS as compared to the controls.

Kilic et al in 2011 conducted a study to evaluate the best and low-cost treatment strategy for Cardiovascular risk in obese and nonobese patients who are suffering from PCOS. It was concluded that with the use of metformin, metabolic and hormonal parameters were improved. However, it was reported that the use of oral contraceptives in obese and normal weight patients with PCOS and Diabetes increases ADMA and hs – CRP levels.

MATERIALS AND METHODS

01.Subjects: - Women visiting gynecology Out Patient with primary complaints of irregular mensural and or infertility will be evaluated. Every selected subject will undergo detailed clinical examination and sonography which includes the presence and estimation of size of polycystic ovaries on ultrasound. Subjects will be recruited from different hospitals across Kashmir valley. The inclusion of PCOS patients shall be done on the basis of Rotterdam (2003) Criteria.

02. Biochemical and hormonal profiling shall be done using commercially available Kits.

03. Clinical and anthropometric measurements: -Anthropometric measurements will be done on the basis of a series of quantitative measurements of the bone, muscle and adipose tissues. These parameters are used to access the composition of the body. The core elements used for anthropometry are weight, height, body circumferences (hip, limbs and waist), Body Mass Index (BMI) and skinfold thickness also. Consideration and measurement of these parameters are important because they represent diagnostic criteria for the obesity. The risk factors associated with obesity are Hypertension Cardiovascular diseases, Diabetes Mellitus and some others.

BMI is calculated by weight divide by height and is measured in kg/m2. For lean female with PCOS, BMI ≥18, for normal 18-25, for overweight 25-30 and for obese patients the BMI will be <30.Serum levels of HS-CRP, IL-6 and TNFα lipid and hormone profiles will be measured.

04. Sample collection: -The blood samples required for basic biochemical and hormonal investigations will be collected under sterile conditions, in clot activator tubes. Serum will be collected for some tests which require the use of serum. Some require plasma and other tests require anti-coagulated whole blood.

05. Investigations and essay: It will require the following tests:

OGTT (oral glucose tolerance test), KFT, LFT, Cholesterol, Testosterone, TSH, TH and T3. The basic biochemical parameters (OGTT, KFT and lipid profile) will be estimated on semi-automated analyzer by using ERBA diagnostic non-hein-Gmbh kits.

Hormonal analysis LH, FSH, TSH, T4 will be done by using chemiluminesense Immunoassay. The sampling for hormonal analysis will be done on 2nd to 7th day of the follicular phase of menstrual cycle.

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06. ELISA:-Enzyme Linked Immunosorbent Assay technique, it is plate based technique used for quantifying and detecting soluble substances such as peptides, proteins, antibodies and enzymes. Levels of insulin, adenopectin and interleukins IL will be estimated BIO-RAD by using ELISA kits according to the respective protocols**.**

07. Statistical Analysis:-Statistical analysis will be carried out to compare the mean levels of cardiovascular laboratory parameters between lean and obese patients with PCOS. To assess the relationship between obesity indices and these laboratory parameters suitable software will be used.

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